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Microtubule Assembly: Catastrophe Factors to the Rescue

Dispatch

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Two new studies have shown how regulation of microtubule dynamics by members of the kinesin superfamily may guard against errors in spindle assembly and chromosome segregation.

It can be convenient to think of the mitotic spindle as a muscle that can both push and pull. As in a muscle, forces in the spindle can be generated by motor proteins that slide filaments relative to each other. The microtubule filaments that make up the cytoskeletal framework in spindles, however, can also generate force through their polymerization and depolymerization [1]. In fact, these polymerization dynamics can be fast enough to replace half the tubulin in spindles every 100 seconds [1]. The metaphase spindle has therefore been described as being in a state of 'dynamic order', resulting from the superposition of turnover due to microtubule dynamic instability and polewards flux, where the movement of the entire microtubule lattice towards spindle poles is coupled to assembly at microtubule plus-ends and disassembly at minus-ends [2]. Classic studies used drugs and changes in pressure and temperature to examine the contribution of microtubule dynamics to spindle assembly and function [1]. The recent discovery that proteins in the kinesin superfamily can directly regulate microtubule dynamics has been an important advance in our understanding of molecular mechanisms underlying the complex behaviors of spindle microtubules. Two recent studies [3,4] have now revealed how errors in spindle morphogenesis and chromosome segregation may be averted by kinesins regulating microtubule dynamics at different sites within cells.

In recent years, two families of kinesins have been identified that act by destabilizing microtubules. One group of molecules is the Kin I family of motor proteins. Members of this family were first identified in human cells [5,6], but subsequent work in the *Xenopus* system and *in vitro* was required to elucidate their novel function [7,8]. Unlike traditional kinesins, which can move along microtubules, Kin I kinesins use ATP hydrolysis to increase the incidence of 'catastrophes', where microtubules change from a state of polymerization to one of depolymerization. Studies in *Xenopus* cell free extracts and mammalian cells have shown that Kin I kinesins are essential for normal mitotic spindle function [7]. Either inhibition or overexpression of Kin I kinesins leads to mitotic defects, such as aberrant spindle morphology, changes in microtubule polymer levels and problems with chromosome congression.

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A second group of kinesins that can regulate microtubule dynamics is the Kip3 family, first characterized in the budding yeast *Saccharomyces cerevisiae* [9]. Mutations in these motors in yeast result in mitoses with very long microtubules, a phenotype that can be rescued by microtubule-destabilizing compounds [10]. While there is currently no direct evidence that Kip3 kinesins have microtubule destabilizing activity, it is reasonable to infer that they are functional yeast orthologs of the Kin I kinesins, given the sequence similarities between the two families [11] and the phenotype associated with their loss of function.

In their recent study, Goshima and Vale [4] used RNA interference (RNAi) to carry out a comprehensive analysis of the contributions of all *Drosophila* kinesins to mitosis in Schneider (S2) tissue culture cells. Analysis of cells lacking the *Drosophila* Kin I kinesin Klp10a showed that most had monopolar spindles with elongated microtubules; some had bipolar spindles that were longer than those in control cells. Both of these phenotypes are consistent with previous studies of Kin I kinesins in other cell types [12].

But something less expected was revealed upon closer examination of the bipolar spindles in the Kin I-depleted cells: staining for γ-tubulin showed that a significant percentage of the bipolar spindles contained centrosomes at only one of the two spindle poles. Such 'monoastral bipolar spindles' (Figure 1a) have previously been observed in non-meiotic *Drosophila* cells [13] and mammalian cells in which one centrosome had been removed by laser microsurgery [14]. The cells with monoastral bipolar spindles were observed undergoing anaphase [4], consistent with the view that the acentrosomal poles are functional and the polarity of the microtubules is correct, with minus-ends at the poles. Real-time microscopy of cells expressing GFP-tubulin, after Kin I RNAi, revealed that monopolar spindles with elongated microtubules formed immediately after nuclear envelope breakdown, at the start of mitosis. Over time, the monopolar spindles underwent a direct transition into bipolar structures with chromosomes at the center. Silencing of the *Drosophila* Kip3 homolog led to a similar, though not identical, phenotype.

It remains unclear where, in a Kin I or Kip3 depleted cell, the microtubules that are organized into the acentrosomal pole come from. One possibility is that microtubules are nucleated at the centrosome, released and then organized by motor proteins. A more intriguing possibility is that microtubule destabilizing kinesins suppress centrosome-independent pathways for microtubule nucleation, maintaining the kinetic advantage centrosomes have over other microtubule nucleation pathways [15]. The ability of microtubules to nucleate at kinetochores has been demonstrated *in vitro* [16] and under some circumstances *in vivo* [17]. The appearance of microtubules at sites proximal to chromosomes and in the cytoplasm during mitosis, and

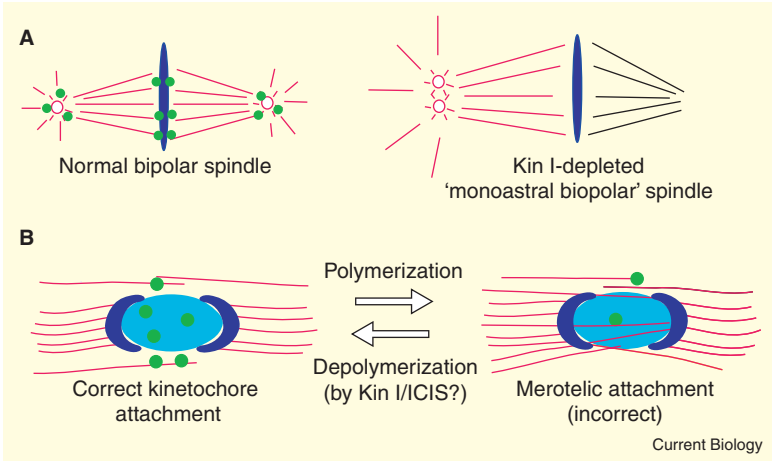

Figure 1. Kin I functions in spindle assembly and at kinetochores.

(A) A normal bipolar spindle with Kin I (green) localized to the kinetochores and centrosomes, and a Kin I-depleted 'monoastral bipolar' spindle with an acentrosomal pole composed of microtubules of unknown origin (black). (B) A model adapted from the work of Ohi *et al.* [3]. KinI–ICIS complexes (green) localized to the inner centromere (light blue) might prevent improper kinetochore–microtubule attachments by destabilizing microtubules that extend past the kinetochore.

their incorporation into spindles, has also been directly observed [18,19].

How are these kinesins regulated to allow microtubule assembly at some sites within cells, such as centrosomes, but not at others? Recent work by Ohi *et al.* [3] suggests one solution to this problem. In the course of a biochemical screen for microtubule-associated proteins (MAPs), Ohi *et al.* [3] identified a protein they have named ICIS, for inner centromere Kin I stimulator; antibody inhibition of ICIS function in *Xenopus* cell-free extracts resulted in a dramatic increase in the level of microtubule polymer. The similarity of the observed phenotype to that caused by loss of Kin I function [7] in these cell extracts suggested that ICIS might indeed regulate microtubule-destabilizing kinesins. Consistent with this hypothesis, it was found that ICIS interacts with Kin I kinesin and is capable of stimulating the microtubule destabilizing activity of Kin I kinesin *in vitro* [3].

Kin I kinesin has been shown to be very active *in vitro* [8]. Why would ICIS be needed to stimulate its activity? The answer may lie in the finding that INCENP and Aurora B interact biochemically with ICIS and Kin I, and these proteins are all targeted to the inner centromere, near sister kinetochores. During cell division, sister kinetochores must attach to microtubules from opposite spindle poles. Errors in chromosome segregation can occur if the kinetochores attach incorrectly — for example, if their attachment is 'syntelic', where both sister kinetochores on a chromosome attach to a single pole, or 'merotelic', where one kinetochore attaches to two poles — and these attachments are not corrected before anaphase. Recent studies have shown that the Aurora B kinase pathway is part of the mechanism that corrects improper chromosome attachments [20]. It is possible that the Aurora kinase may regulate Kin I kinesins at kinetochores via ICIS to sever only improper attachments to the spindle (Figure 1B). Looking for direct evidence for such a regulation is an exciting direction for further research.

Kin I kinesins have been shown to regulate the dynamic instability of spindle microtubules [7]. But it is unclear what role they play in polewards flux, the other key mechanism contributing to spindle microtubule dynamicity and force production. An important challenge for future research is to determine whether mechanisms similar to those that may operate at kinetochores and during spindle assembly are also involved in regulating microtubule destabilizing activities at spindle poles to drive polewards flux.

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